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Total Synthesis of (+)-Prunustatin A: Utility of Organotrifluoroborate-Mediated Prenylation and Shiina MNBA Esterification and Macrolactonization To Avoid a Competing Thorpe−Ingold Effect Accelerated Transesterification

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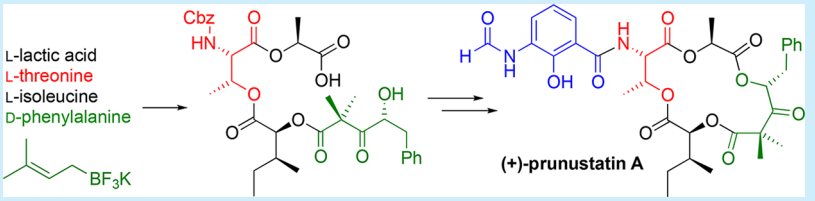
Supporting Information



ABSTRACT: A convergent total synthesis of (+)-prunustatin A is described through the assembly of two key fragments and a macrolactonization. Shiina MNBA couplings were used for the formation of each of the four ester bonds in the tetralactone ring, including the key macrocyclization which was essential to minimize competing Thorpe−Ingold accelerated transesterification. Other key steps included an organoboron-based prenylation using potassium prenyltrifluoroborate and a carbonyldiimidazole-mediated coupling to form the salicylamide.

Prunustatin A (**1**) is a natural product isolated in 2005 from a soil sample collected on the volcanic island of Kumajima in Okinawa, Japan, and determined by Shin-ya to be a potent anticancer agent against hypoxic fibrosarcoma cells.[1] It is produced by the bacterial species *Streptomyces violaceoniger*, which belongs to a family of biocontrol agents used against phytopathogenic fungi.[2] Structurally, **1** is a member of the antimycin depsipeptide class of compounds[3] that includes (+)-SW-163A **2**,[4] JBIR-06,[1e] JBIR-52,[5] neoantimycin **3**,[6] the parent antimycin family of compounds **4**,[6b,7] and respirantin **5**[8] (Figure 1). They share an aromatic 3-formamidosalicylate headgroup attached via an amide bond to an L-threonine unit embedded within a di-, tri-, or tetralactone macrocyclic ring (9-, 12-, 15-, or 18-membered). Other antimycin depsipeptides incorporate different aromatic head groups, with the entire family related through a nonribosomal peptide synthetase (NRPS)-polyketide synthase (PKS) biosynthetic pathway. Over the years, there has been significant interest in this class of depsipeptides due to their anticancer, antifungal, and immunosuppressant properties. The anticancer properties of (+)-prunustatin A are believed to stem from its ability to downregulate the expression of GRP78, which is a glucose-regulated molecular chaperone that plays an important role in the survival of tumors subjected to chemotherapy. The inhibition of GRP78 expression may be a secondary effect of blocking the mitochondrial ATP production.[9]

The structural motifs present in these molecules pose numerous synthetic challenges, especially the formation of the macrocyclic rings. Our previous work in this class of natural

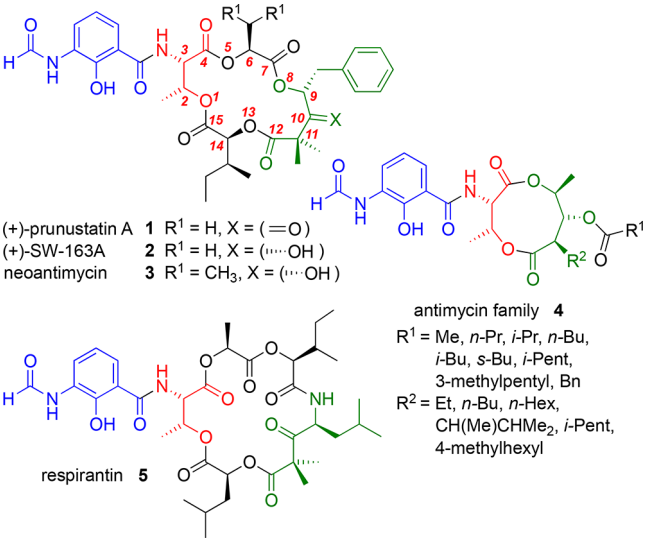


Figure 1. Structures of (+)-prunustatin A (**1**) and related natural products **2**−**5** belonging to the antimycin class of compounds.

products has included the synthesis of antimycin A_{1b} (2014)[10] as well as respirantin and kitastatin (2014).[11] In the latter case, the presence of a lactam group permitted the use of a macrolactamization approach using HATU, whereas Shiina's

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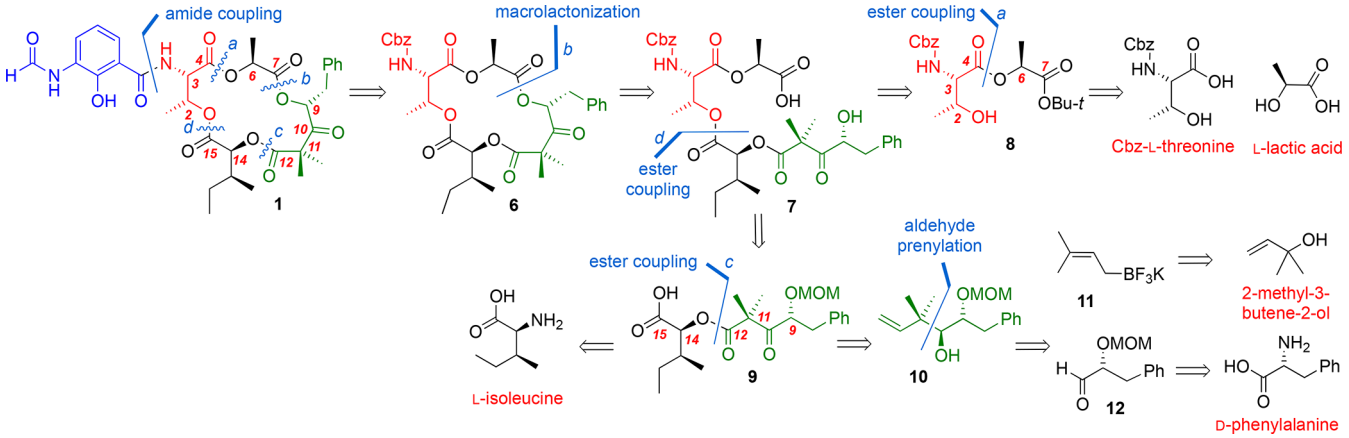
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Retrosynthesis of (+)-Prunustatin A (1)



reagent (MNBA: 2-methyl-6-nitrobenzoic anhydride)[12] was required for the challenging macrolactonization step needed to form the 9-membered dilactone ring present in antimycin. We now report a total synthesis of (+)-prunustatin A that addresses some of the problems encountered by other groups in the total synthesis of **1**.

The major challenges associated with the synthesis of (+)-prunustatin A (**1**) are the formation of the 15-membered tetralactone ring, the requisite seco acid precursor, and the *β*-keto-*α*-*gem*-dimethyl ester functionality, which is also found in JBIR-06, respirantin, kitastatin, and the subsequent attachment of the 3-formamidosalicylate headgroup. Each of the existing approaches to **1** by Kawanishi,[13] Usuki,[14] and Hale[15] have involved linear strategies for the formation of triester precursors, followed by macrolactonization through bond *a* or *c* formation (Scheme 1). In 2014, Kawanishi achieved the first total synthesis of **1** using a macrocyclization to form bond *a* in 60% yield using MNBA/DMAP at 50 °C. Attempts to remove a benzyl group from the O8 position of a C9−C15 ester precursor revealed a propensity for an undesired transesterification at the C12 position with concomitant fragmentation to a butyrolactone as a result of the Thorpe−Ingold effect of the C11 *gem*-dimethyl group. Usuki and Hale encountered problems in their attempts to achieve macrolactonization through bond *b* or *d* formation, respectively (Scheme 1). Hale's initial approaches utilizing a bond *d* macrocyclization led only to diastereomers, thus necessitating a bond *a* macrocyclization strategy. Hale also reported that application of Kawanishi's high-temperature conditions occurred with problems of lower yields and reproducibility and, consequently, resorted to a reoptimization of the MNBA/DMAP conditions at room temperature and at "ultrahigh dilution" to form the desired macrolactone in 42% yield along with some dimerized products. Usuki's initial approach utilizing a bond *b* macrocyclization strategy was thwarted because of undesired butyrolactone formation similar to that encountered by Kawanishi. As a result, Usuki employed a strategy employed in Pettit's respirantin **5** synthesis[8b] involving macrocyclization of a less sterically encumbered substrate lacking the *gem*-dimethyl group. Thus, macrolactonization was achieved through transesterification of a *β*-ketoester leading to bond *c* formation in 88% yield using 20 equiv of $CuSO_4$ in toluene at reflux. This protocol requires a subsequent *α*-methylation step to yield the gem dimethyl substituted group in 62% yield.

In summary, Kawanishi employed a linear sequence of ester bond *c*, *d*, and *b* formation and macrocyclization of bond *a*, Hale employed a linear sequence of ester bond *b*, *c*, and *d* formation and macrocyclization of bond *a*, while Usuki employed a linear sequence involving ester bond *a*, *d*, and *b* formation, macrocyclization of bond *c*, and subsequent C11 *gem*-dimethyl group introduction. Based upon this precedent, we were particularly interested in seeing whether a fully convergent synthesis of **1** might be possible and whether the troublesome *gem*-dimethyl-promoted butyrolactone formation could be avoided using an approach based upon a bond *b* macrocyclization of a seco acid substrate that incorporated a pre-existing C11 *gem*-dimethyl group. The requisite fragments would also need to be synthesized from readily available precursors using a convergent strategy and allow for the late-stage incorporation of different aromatic headgroups, preferably avoiding the use of a preformed O-protected 3-formamidosalicylic acid as a coupling partner.

The quasi-symmetric nature of the tetralactone ring in **1** is such that there are four possible convergent strategies for its formation. An approach involving initial C6−C12 and C14−C4 fragment synthesis through ester bond *b* and *d* formation would require subsequent ester bond *a* and *c* formation. This is undesirable due to the very hindered nature of the ester bond *c* and the possibility of competitive decarboxylation of any C9−C12 *β*-keto-*α*-*gem*-dimethyl ester precursor fragment. Alternatively, the convergent synthetic approach involving a late-stage combination of bond *d* and *b* formation would allow for the inherently more challenging ester bond *c* formation to be incorporated more straightforwardly for the formation of the "southern" fragment **9**. Therefore, we chose a strategy in which the key seco acid intermediate **7** required for the lactone bond *b* formation would be formed through an intermolecular ester bond *d* disconnection between two similar size fragments, **8** (C2−C7) and **9** (C9−C15) which, in turn, would be accessed through ester bond *a* and *c* formation, respectively, and use a prenyl trifluoroborate salt **11** to introduce the C11 *gem*-dimethyl group in **9** (Scheme 1).

The "northern" fragment **8** synthesis started with the formation of **15** using a modified three-step protocol of Kirschning (Scheme 2).[16] Acetylation of L-lactic acid to give **13** followed by an EDC-mediated *tert*-butyl protection to give **14** in 84% yield (EDC offered yield and reaction time improvement over the traditional Steglich esterification used in the original sequence). Cleavage of the acetyl group under basic

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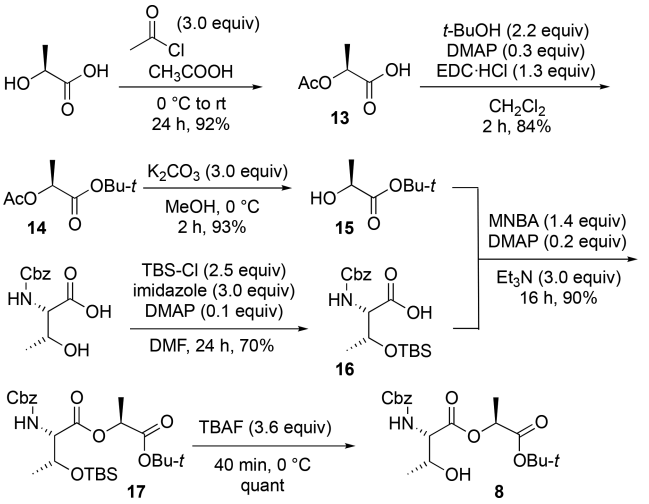
Org. Lett. XXXX, XXX, XXX−XXX

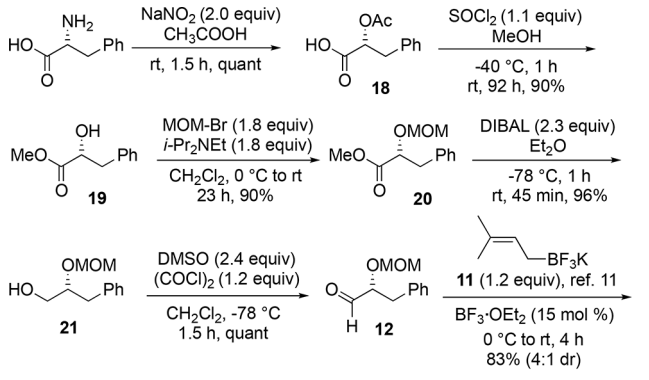
Scheme 2. Synthesis of "Northern" Fragment 8

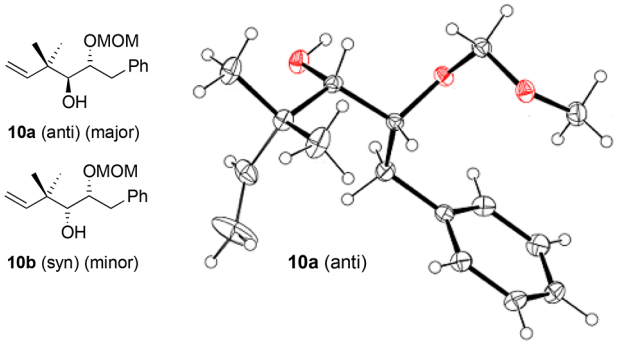


conditions yielded alcohol **15** in 93% yield. The synthesis of acid **16** was achieved by TBS protection of commercially available Z-Thr.[17] The ester coupling between **16** and **15** using MNBA, DMAP, and triethylamime afforded **17** in 90% yield. Finally, TBAF deprotection of the TBS group yielded **8** in quantitative yield.[18]

The synthesis of the "southern" fragment required the use of the MOM-protected α-hydroxy aldehyde **12** (Scheme 3). A diazotization of D-phenylalanine in acetic acid occurs with overall retention of stereochemistry[19] to give the acetate **18**, which upon prolonged treatment with thionyl chloride in methanol led to **19**. MOM protection to yield **20**[20] followed by ester group reduction with DIBAL and Swern oxidation gave aldehyde **12** in high yield.[21] Given the possible epimerization

Scheme 3. Synthesis of Aldehyde 12 and Prenylation Reaction

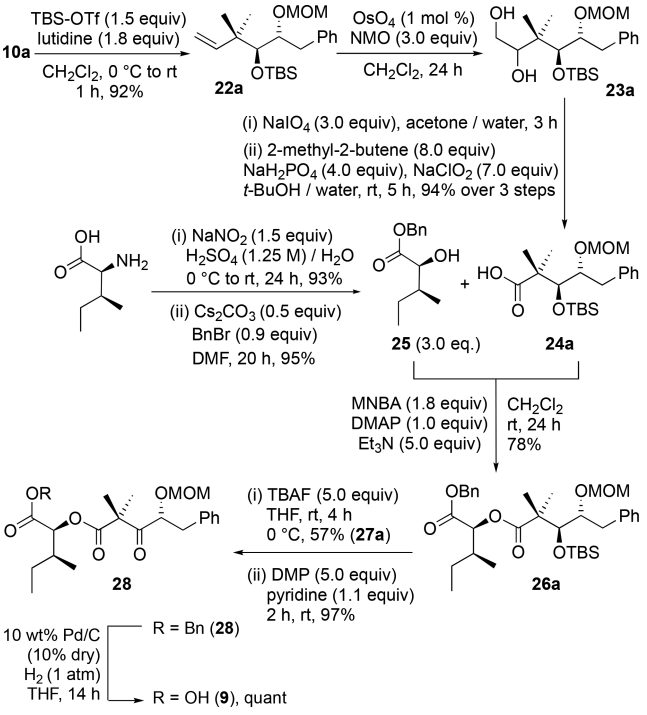




issues, **12** was used immediately or stored at −78 °C for up to a week with enantiomeric purity confirmed by $[\alpha]_D$ determinations. The prior success that we had achieved using organotrifluoroborate salts in the synthesis of **5** encouraged us to attempt an analogous approach for the introduction of the *gem*-dimethyl group. Reaction of the freshly prepared aldehyde **12** with 3.0 equiv of prenyltrifluoroborate salt **11**[11] for 3 days at room temperature using Montmorillonite K10 clay based activation afforded **10** as a 5:1 mixture of anti and syn diastereomers (**10a** and **10b**) with a total yield of 80%. The salt **11** is a stable, white solid that can be stored for a prolonged time with minimal decomposition offering a convenient alternative to organometallic prenylations of aldehydes and ketones under mild conditions.[11] The reaction time and the amount of the salt **11** required could be reduced with the use of 15 mol % $BF_3{\cdot}OEt_2$ as a catalyst, giving **10** as a 4:1 mixture of diastereomers in 83% total yield.[22] The structure of the major diastereomer was confirmed by X-ray crystallography as having an anti relationship consistent with a Cornforth transition-state model.[10]

Even though both diastereomers of **10** converge onto the same product **28** during the later oxidation step, the alcohols were separated by column chromatography (Scheme 4). TBS

Scheme 4. Synthesis of "Southern" Fragment 9



protection of the major anti diastereomer **10a** with TBS-OTf afforded **22a**, which upon dihydroxylation with OsO_4, oxidative cleavage with $NaIO_4$, and Pinnick oxidation yielded carboxylic acid **24a** (94% over three steps). The benzyl ester protected coupling partner **25** was also synthesized using a diazotization approach from L-isoleucine in two steps. The subsequent ester coupling was particularly challenging due to the steric hindrance of the carboxylic acid **24a**, and the use of Shiina conditions using 3.0 equiv of the secondary alcohol coupling partner **25** was necessary to afford ester **26a** in good yields. After TBAF deprotection of the TBS group, oxidation with DMP gave ketone **28**. An analogous sequence using the

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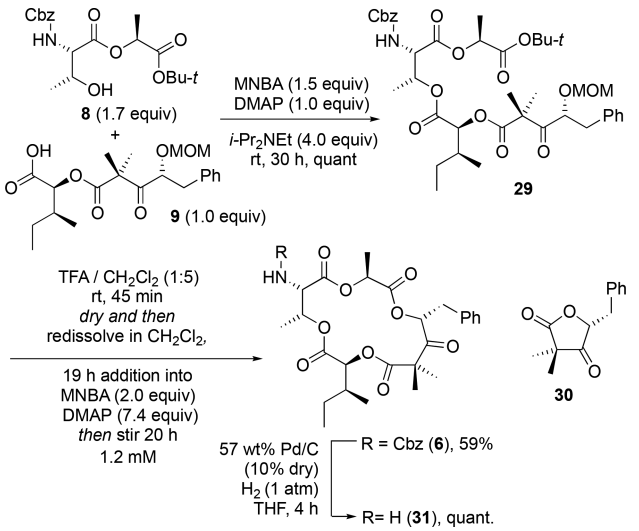
Org. Lett. XXXX, XXX, XXX−XXX

syn diastereomer **10b** also gave **28** in similar yields. Removal of the benzyl ester protecting group from **28** using H_2 and Pd/C occurred in quantitative yield to give acid **9**.

The coupling of the two fragments was once again achieved using Shiina MNBA coupling conditions to give **29** in quantitative yield (Scheme 5). The choice of acid-labile

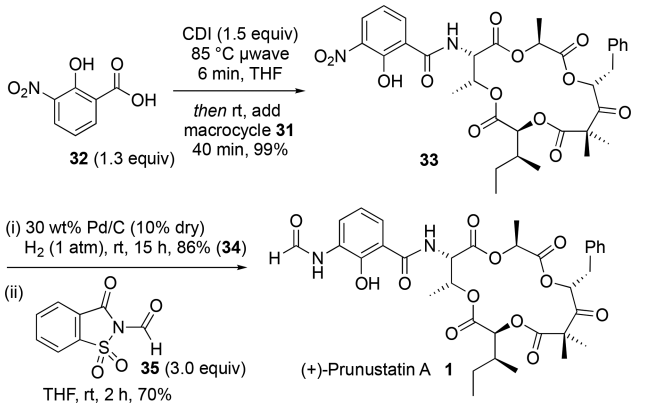
Scheme 5. Fragment Coupling and Double Deprotection/ Macrocyclization



protecting groups in compound **29** allowed for the sequential removal of the MOM and *t*-Bu ester groups using TFA in a "one-pot" fashion.[23] After thorough drying of the reaction mixture, the seco acid **7** was obtained in 82−90% purity (NMR). The remainder of the mixture was the butyrolactone **30** and associated degraded seco acid. This degradation is facilitated by the Thorpe−Ingold effect as noted in related, more problematic, reactions in the Kawanishi[13] and Usuki syntheses of **1**,[14] as well as in Pettit's respirantin synthesis in which a pyrrolidinone equivalent of the lactone was formed.[8b] Seco acid **7**, obtained from **29** with TFA, was subjected to macrocyclization under slow-addition, high-dilution conditions (1.2 mM) with MNBA/DMAP to afford the macrocycle **6** in 59% yield over two steps. To minimize the formation of **30** during the double deprotection, TFA was removed using cold $CHCl_3$ (11−14 °C), and the reaction mixture was dried and used immediately without exposure to temperature >20 °C to prevent further degradation to **30**. This and proper handling of the reagents allows for macrocyclization through disconnection *b* for the first time with improved efficiency and in good yield. In comparison previous macrocycle formations were performed over ester bond *a* (Hale: 42% yield, Kawanishi: 60% yield) and ester bond *c* (Usuki: 88% yield) followed by alkylation (62% yield for α-methylation step; 55% yield over two steps). The key amine macrocycle **31** was then obtained in quantitative yield after Pd/C deprotection.

The 3-formamidosalicylate headgroup is typically coupled to the amine group of the macrocycle in syntheses of **1** and other antimycin analogues using a 3-formamidosalicyic acid protected at the phenolic OH. The synthesis of these precursors usually requires a few steps including a particularly harsh N-formylation reaction with sometimes difficult purification,[10] followed by benzyl protection of the phenolic oxygen, amide coupling with the macrocycle using HATU or EDCI, and a deprotection step.[6−8,13−15] In order to avoid these problems, we used an indirect approach in which unprotected 3-nitrosalicylic acid **32** was first activated with CDI (1,1′-carbonyldiimidazole) under microwave conditions for 6 min at 85 °C, cooled to room temperature, and then coupled with the macrocycle **31** in 40 min to afford **33** in 99% yield using a simple silica plug purification (Scheme 6).

Scheme 6. Completion of the Synthesis of (+)-Prunustatin A (1)



Reduction of the nitro group yielded aniline **32** in 86% yield and immediate, mild formylation using *N*-formylsaccharin **35** (Cossy's protocol)[24] yielded (+)-prunustatin A (**1**) in 70% yield. This efficient three-step protocol potentially allows for late-stage functionalization of either the phenol or aniline functionalities on the headgroup.

In conclusion, consideration of the quasi-symmetric nature of the tetralactone ring of (+)-prunustatin A, one of the most complex cyclic polylactones known, allowed for a convergent approach to its total synthesis using Shiina MNBA coupling conditions for each of the ester bonds, a CDI-activated amide bond formation, and an *N*-formylsaccharin-mediated formylation. The requisite α-hydroxy acid fragments were synthesized through α-amino acid diazotization chemistry, and the C11 *gem*-dimethyl group of the β-keto-α-*gem*-dimethyl ester motif installed using an organoboron-based approach using a stable prenyl trifluoroborate salt. An efficient double-deprotection/ macrolactonization allowed for ring closure to occur at an ester bond that had previously been reported to be unsuccessful.[14] Further studies on this class of molecules and analogues will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02396.

Full experimental details and characterization data for all compounds including ^1H and ^{13}C NMR spectral data (PDF)

Accession Codes

CCDC 1858839 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cam-

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Notes

The authors declare no competing financial interest.

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